

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising a mutant peptide sequence, wherein the mutant peptide sequence encodes an O-linked glycosylation site that does not exist in a wild-type polypeptide corresponding to the isolated polypeptide.

2. The polypeptide of claim 1, wherein the polypeptide is a G-CSF polypeptide.

3. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises a mutant peptide sequence with the formula of M^1X_nTPLGP or $M^1B_oPZ_mX_nTPLGP$, and wherein

the superscript denotes the position of the amino acid in the wild-type G-CSF amino acid sequence (SEQ ID NO:3), the subscripts n and m are integers selected from 0 to 3, and

at least one of X and B is Thr or Ser, and

when more than one of X and B is Thr or Ser, the identity of these moieties is independently selected, and

Z is selected from glutamate, or any uncharged amino acid.

4. The mutant G-CSF polypeptide of claim 3, wherein the mutant peptide sequence is selected from the sequences consisting of MVTPLGP, MQTPLGP, MIATPLGP), MATPLGP, MPTQGAMPLGP, MVQTPLGP, MQSTPLGP, MGQTPLGP, MAPTSSSPLGP, and MAPTPLGPA.

5. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises a mutant peptide sequence with the formula of $M^1TPX_nB_oO_rP$ wherein

the superscript denotes the position of the amino acid in SEQ ID NO:3, and

the subscripts n, o, and r are integers selected from 0 to 3, and

at least one of X, B and O is Thr or Ser, and

when more than one of X, B and O is Thr or Ser, the identity of these moieties is independently selected.

1 6. The polypeptide of claim 5, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: MTPTLGP, MTPTQLGP, MTPTSLGP,
3 MTPTQGP, MTPTSSP, M¹TPQTP, M¹TPTGP, M¹TPLTP, M¹TPNTGP, MTPLGP (G-
4 CSF mut #4), M¹TPVTP, M¹TPMVTP, and MT¹P²TQGL³G⁴P⁵A⁶S⁷.

1 7. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of LGX⁵³B_oLGI
3 wherein
4 the superscript denotes the position of the amino acid in the wild type G-CSF
5 amino acid sequence (SEQ ID NO: 3), and
6 X is histidine, serine, arginine, glutamic acid or tyrosine, and
7 B is either threonine or serine, and
8 o is an integer from 0 to 3.

1 8. The polypeptide of claim 7, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: LGHTLGI, LGSSLGI, LGYSLGI, LGESLGI,
3 and LGSTLGI.

1 9. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of P¹²⁹Z_mJ_qO_rX_nPT
3 wherein
4 the superscript denotes the position of the amino acid in the wild type G-CSF
5 amino acid sequence (SEQ ID NO. 3),
6 Z, J, O and X are independently selected from Thr or Ser, and
7 m, q, r, and n are integers independently selected from 0 to 3..

1 10. The polypeptide of claim 9, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: P¹²⁹ATQPT, P¹²⁹TLGPT, P¹²⁹TQGPT,
3 P¹²⁹TSSPT, P¹²⁹TQGAPT, P¹²⁹NTGPT, PALQPTQT, P¹²⁹ALTPT, P¹²⁹MVTPT,
4 P¹²⁹ASSTPT, P¹²⁹TTQP, P¹²⁹NTLP, P¹²⁹TLQP, MAP¹²⁹ATQPTQGAM, and
5 MP¹²⁹ATTQPTQGAM.

1 11. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of PZ_mU_sJ_qP⁶¹O_rX_nB_oC
3 wherein

4 the superscript denotes the position of the amino acid in the wild type G-CSF
 5 amino acid sequence (SEQ ID NO. 3),
 6 at least one of Z, J, O, and U is selected from threonine or serine, and
 7 when more than one of Z, J, O and U is threonine or serine, each is
 8 independently selected, and
 9 m, s, q, r, n, and o are integers independently selected from 0 to 3.

1 12. The polypeptide of claim 11, wherein the mutant peptide sequence is
 2 selected from the sequences consisting of: P⁶¹TSSC, P⁶¹TSSAC, LGIPTA P⁶¹LSSC,
 3 LGIPTQ P⁶¹LSSC, LGIPTQG P⁶¹LSSC, LGIPQT P⁶¹LSSC, LGIPTS P⁶¹LSSC, LGIPTS
 4 P⁶¹LSSC, LGIPTQP⁶¹LSSC, LGTPWAP⁶¹LSSC, LGTPFA P⁶¹LSSC, P⁶¹FTP, and
 5 SLGAP⁵⁸TAP⁶¹LSS.

1 13. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
 2 a mutant peptide sequence with the formula of $\emptyset_a G_p J_q O_r P^{175} X_n B_o Z_m U_s \Psi_t$
 3 wherein
 4 the superscript denotes the position of the amino acid in the wild type G-CSF
 5 amino acid sequence (SEQ ID NO. 3),
 6 at least one of Z, U, O, J, G, \emptyset , B and X is threonine or serine, and when more
 7 than one of Z, U, O, J, G, \emptyset , B and X are threonine or serine, they are
 8 independently selected; \emptyset is optionally R, and G is optionally H; the symbol Ψ
 9 represents any uncharged amino acid residue or glutamate and
 10 a, p, q, r, n, o, m, s, and t are integers independently selected from 0 to 3..

1 14. The polypeptide of claim 13, wherein the mutant peptide sequence is
 2 selected from the sequences consisting of: RHLAQTP¹⁷⁵, RHLAQTP¹⁷⁵,
 3 QP¹⁷⁵TQGAMP, RHLAQTP¹⁷⁵AM, QP¹⁷⁵TSSAP, QP¹⁷⁵TSSAP, QP¹⁷⁵TQGAMP,
 4 QP¹⁷⁵TQGAM, QP¹⁷⁵TQGA, QP¹⁷⁵TVM, QP¹⁷⁵NTGP, and QP¹⁷⁵QTLF.

1 15. The polypeptide of claim 2, comprises a mutant peptide sequence
 2 selected from the sequences P¹³³TQTAMP¹³⁹, P¹³³TQGTMP, P¹³³TQGTNP,
 3 P¹³³TQGTLP, and PALQP¹³³TQTAMPA.

1 16. The polypeptide of claim 1, wherein the polypeptide is an hGH
 2 polypeptide.

1 17. The polypeptide of claim 16, wherein the mutant peptide sequence
2 comprises a sequence selected from: M¹APTSSPTIPL⁷SR⁹ and DGSP¹³³NTGQIFK¹⁴⁰

1 18. The polypeptide of claim 15, wherein the hGH polypeptide comprises
2 a mutant peptide sequence with a formula of P¹³³JXBOZUK¹⁴⁰QTYS, and
3 wherein

4 the superscript denotes the position of the amino acid in the wild type hGH
5 amino acid sequence (SEQ ID NO: 20), and

6 J is selected from threonine and arginine;

7 X is selected from alanine, glutamine, isoleucine, and threonine;

8 B is selected from glycine, alanine, leucine, valine, asparagine, glutamine, and
9 threonine;

10 O is selected from tyrosine, serine, alanine, and threonine;

11 Z is selected from isoleucine and methionine; and

12 U is selected from phenylalanine and proline.

1 19. The polypeptide of claim 18, wherein the mutant peptide sequence is
2 selected from the group consisting of PTTGQIFK, PTTAQIFK, PTTLQIFK,
3 PTTLYVFK, PTTVQIFK, PTTVSIFK, PTTNQIFK, PTTQQIFK, PTATQIFK,
4 PTQGQIFK, PTQGAIFK, PTQGAMFK, PTIGQIFK, PTINQIFK, PTINTIFK,
5 PTILQIFK, PTIVQIFK, PTIQQIFK, PTIAQIFK, P¹³³TTTQIFK¹⁴⁰QTYS, and
6 P¹³³TQGAMPK¹⁴⁰QTYS.

1 20. The polypeptide of claim 15, wherein the hGH polypeptide comprises
2 a mutant peptide sequence with a formula of P¹³³RTGQIPTQBYS
3 wherein

4 the superscript denotes the position of the amino acid in the wild type hGH
5 amino acid sequence (SEQ ID NO:20), and

6 B is selected from alanine and threonine.

1 21. The polypeptide of claim 20, wherein the mutant peptide sequence is
2 selected from the group consisting of PRTGQIPTQTYS and PRTGQIPTQAYS.

1 22. The polypeptide of claim 16, wherein the hGH polypeptide comprises
2 a mutant peptide sequence with a formula of L¹²⁸XTBOP¹³³UTG

3 wherein
 4 superscripts denote the position of the amino acid in the wild-type hGH amino
 5 acid sequence; and wherein
 6 X is selected from glutamic acid, valine and alanine;
 7 B is selected from glutamine, glutamic acid, and glycine;
 8 O is selected from serine and threonine; and
 9 U is selected from arginine, serine, alanine and leucine

1 23. The mutant hGH polypeptide of claim 22, wherein the mutant peptide
 2 sequence is selected from the group consisting of: LETQSP¹³³RTG, LETQSP¹³³STG,
 3 LETQSP¹³³ATG, LETQSP¹³³LTG, LETETP¹³³R, LETETP¹³³A, LVTQSP¹³³RTG,
 4 LVTETP¹³³RTG, LVTETP¹³³ATG, and LATGSP¹³³RTG.

1 24. The polypeptide of claim 16, wherein the hGH polypeptide comprises
 2 a mutant peptide sequence with a formula of M¹BPTX_nZ_mOPLSRL
 3 wherein
 4 wherein the superscript denotes the position of the amino acid in the wild type
 5 hGH amino acid sequence (SEQ ID NO:19); and
 6 B is selected from phenylalanine, valine and alanine or a combination thereof;
 7 X is selected from glutamate, valine and proline
 8 Z is threonine;
 9 O is selected from leucine and isoleucine; and
 10 when X is proline, Z is threonine; and
 11 wherein
 12 n and m are integers selected from 0 and 2.

1 25. The polypeptide of claim 24, wherein the mutant peptide sequence is
 2 selected from the group consisting of M¹FPTE IPLSRL, M¹FPTV LPLSRL, and
 3 M¹APTPTIPLSRL.

1 26. The polypeptide of claim 24, wherein the mutant peptide sequence is
 2 M¹VTPTIPLSRL, wherein the superscript 1, denotes the first position amino acid in the
 3 wild type hGH amino acid sequence (SEQ ID NO:19)

1 27. The polypeptide of claim 15, wherein the mutant peptide sequence is
 2 selected from the group consisting of: LEDGSPTTGQIFKQTYS,

3 LEDGSPTTAQIFKQTYS, LEDGSPTATQIFKQTYS, LEDGSPTQGAMFKQTYS,
 4 LEDGSPTQGAIFKQTYS, LEDGSPTQGQIFKQTYS, LEDGSPTTLYVFKQTYS,
 5 LEDGSPTINTIFKQTYS, LEDGSPTTVSIFKQTYS, LEDGSPRTGQIPTQTYS,
 6 LEDGSPRTGQIPTQAYS, LEDGSPTTLQIFKQTYS, LETETPRTGQIFKQTYS,
 7 LVTETPRTGQIFKQTYS, LETQSPRTGQIFKQTYS, LVTQSPRTGQIFKQTYS,
 8 LVTETPATGQIFKQTYS, LEDGSPTQGAMPKQTYS, and LEDGSPTTTQIFKQTYS.

1 28. The polypeptide of claim 1, wherein the polypeptide is an IFN alpha
 2 polypeptide.

1 29. The polypeptide of claim 28, wherein wherein the INF alpha
 2 polypeptide has a peptide sequence comprising a mutant amino acid sequence, and the
 3 peptide sequence corresponds to a region of INF alpha 2 having a sequence as shown in
 4 SEQ NO:22, and wherein the mutant amino acid sequence contains a mutation to a
 5 threonine or serine amino acid at a position corresponding to T¹⁰⁶ of INF alpha 2.

1 30. The polypeptide of claim 29, wherein the IFN alpha polypeptide is
 2 selected from the group consisting of IFN alpha, IFN alpha 4, IFN alpha 5, IFN alpha 6,
 3 IFN alpha 7, IFN alpha 8, IFN alpha 10, IFN alpha 14, IFN alpha 16, IFN alpha 17, and
 4 IFN alpha 21.

1 31. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
 2 IFN alpha polypeptide comprising a mutant amino acid sequence selected from the group
 3 consisting of:

4 ⁹⁹CVMQEERTVTETPLMNADSIL¹¹⁸, ⁹⁹CVMQEEGVETETPLMNADSIL¹¹⁸,
 5 and ⁹⁹CVMQGVGVETETPLMNADSIL¹¹⁸.

1 32. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
 2 IFN alpha 4 polypeptide comprising a mutant amino acid sequence selected from the
 3 group consisting of:

4 ⁹⁹CVIQEVGVETETPLMNVDSIL¹¹⁸, and ⁹⁹CVIQGVGVETETPLMKEDSIL¹¹⁸.

1 33. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
 2 IFN alpha 5 polypeptide comprising a mutant amino acid sequence selected from the
 3 group consisting of:

4 ⁹⁹CMMQEVGVTDTPLMNVDSIL¹¹⁸, ⁹⁹CMMQEVGVTTETPLMNVDISIL¹¹⁸
5 and ⁹⁹CMMQGVGVTDTPLMNVDSIL¹¹⁸.

1 34. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 6 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVMQEVWVTGTPLMNEDSIL¹¹⁸, ⁹⁹CVMQEVGVTTGTPLMNEDSIL¹¹⁸,
5 and ⁹⁹CVMQGVGVTTETPLMNEDSIL¹¹⁸.

1 35. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 7 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVIQEVGVTTETPLMNEDFIL¹¹⁸, and ⁹⁹CVIQGVGVTTETPLMNEDFIL¹¹⁸.

1 36. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 8 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVMQEVGVTTESPLMYEDSIL¹¹⁸, and ⁹⁹CVMQGVGVTTESPLMYEDSIL¹¹⁸.

1 37. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 10 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVIQEVGVTTETPLMNEDSIL¹¹⁸, and ⁹⁹CVIQGVGVTTETPLMNEDSIL¹¹⁸.

1 38. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 14 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVIQEVGVTTETPLMNEDSIL¹¹⁸, and ⁹⁹CVIQGVGVTTETPLMNEDSIL¹¹⁸.

1 39. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 16 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVTQEVGVTEIPLMNEDSIL¹¹⁸, ⁹⁹CVTQEVGVTTETPLMNEDSIL¹¹⁸, and
5 ⁹⁹CVTQGVGVTTETPLMNEDSIL¹¹⁸.

1 40. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 17 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVIQEVGMTETPLMNEDSIL¹¹⁸, ⁹⁹CVIQEVGVETETPLMNEDSIL¹¹⁸, and
5 ⁹⁹CVIQGVGMTETPLMNEDSIL¹¹⁸.

1 41. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 21 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

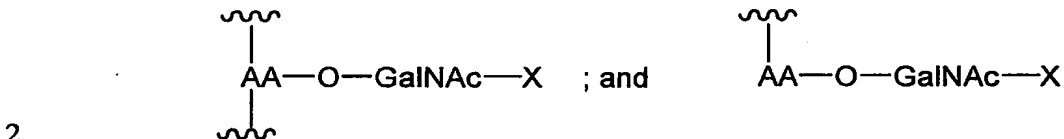
4 ⁹⁹CVIQEVGVETETPLMNVDSIL¹¹⁸, and ⁹⁹CVIQGVGVETETPLMNVDSIL¹¹⁸.

1 42. An isolated nucleic acid encoding the polypeptide of claim 1.

1 43. An expression cassette comprising the nucleic acid of claim 42.

1 44. A cell comprising the nucleic acid of claim 42.

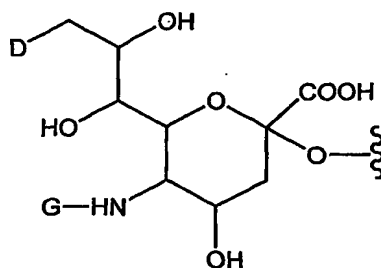
1 45. The polypeptide of claim 1, having a formula selected from:



3 wherein AA is an amino acid side chain that comprises a hydroxyl moiety
4 that is within the mutant peptide sequence; and X a modifying group or a saccharyl moiety.

1 46. The polypeptide according to claim 45, wherein X comprises a group
2 selected from sialyl, galactosyl and Gal-Sia moieties, wherein at least one of said sialyl,
3 galactosyl and Gal-Sia comprises a modifying group.

1 47. The polypeptide according to claim 45, wherein X comprises the
2 moiety:



wherein

D is a member selected from -OH and R^1 -L-NH-;

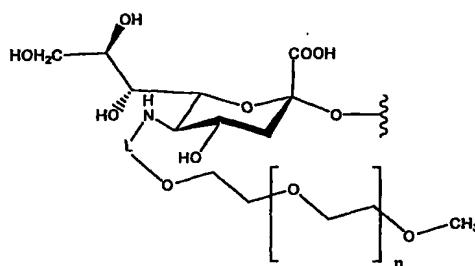
G is a member selected from R^1 -L- and $-C(O)(C_1-C_6)alkyl$;

R^1 is a moiety comprising a member selected a moiety comprising a straight-chain or branched poly(ethylene glycol) residue; and

L is a linker which is a member selected from a bond, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl,

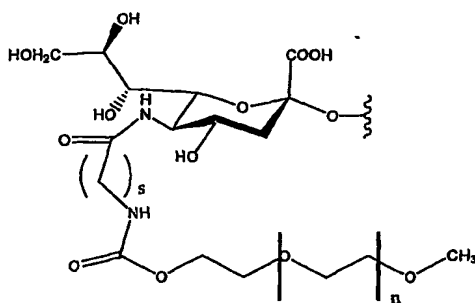
such that when D is OH, G is R^1 -L-, and when G is $-C(O)(C_1-C_6)alkyl$, D is R^1 -L-NH-.

48. The polypeptide according to claim 45, wherein X comprises the structure:



in which L is a substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl group; and n is selected from the integers from 0 to about 500.

49. The polypeptide according to claim 45, wherein X comprises the structure:



wherein s is selected from the integers from 0 to 20.

50. A method for making a glycoconjugate of the polypeptide of claim 1, comprising the steps of:

- (a) recombinantly producing the polypeptide, and
- (b) enzymatically glycosylating the polypeptide with a modified sugar at said O-linked glycosylation site.

51. A pharmaceutical composition of a granulocyte colony stimulating factor (G-CSF) comprising: an effective amount of the polypeptide of claim 2, wherein said polypeptide is glycoconjugated with a modified sugar.

52. The pharmaceutical composition according to claim 51, wherein said modified sugar is modified with a member selected from poly(ethylene glycol) and methoxy-poly(ethylene glycol) (m-PEG).

53. A pharmaceutical composition of human Growth Hormone (hGH) comprising an effective amount of the polypeptide of claim 16, wherein said polypeptide is glycoconjugated with a modified sugar.

54. The pharmaceutical composition according to claim 53, wherein said modified sugar is modified with a member selected from poly(ethylene glycol) and methoxy-poly(ethylene glycol) (m-PEG).

55. A pharmaceutical composition of a granulocyte macrophage colony stimulating factor (GM-CSF) comprising an effective amount of GM-CSF polypeptide comprising a mutant peptide sequence, wherein the mutant sequence comprises an O-linked glycosylation site that does not exist in a wild-type GM-CSF polypeptide, and wherein said polypeptide is glycoconjugated with a modified sugar.

1 56. The pharmaceutical composition according to claim 55, wherein said
2 modified sugar is modified with a member selected from poly(ethylene glycol) and
3 methoxy-poly(ethylene glycol) (m-PEG).

1 57. A pharmaceutical composition of an interferon alpha-2b comprising an
2 effective amount of the polypeptide of claim 28, wherein said polypeptide is
3 glycoconjugated with a modified sugar.

1 58. The pharmaceutical composition according to claim 57, wherein said
2 modified sugar is modified with a member selected from poly(ethylene glycol) and
3 methoxy-poly(ethylene glycol) (m-PEG).

1 59. A method of providing G-CSF therapy to a subject in need of said
2 therapy, said method comprising, administering to said subject an effective amount the
3 pharmaceutical composition of claim 51.

1 60. A method of providing granulocyte macrophage colony stimulating
2 factor therapy to a subject in need of said therapy, said method comprising:
3 administering to said subject an effective amount the pharmaceutical
4 composition of claim 55.

1 61. A method of providing interferon therapy to a subject in need of said
2 therapy, said method comprising:
3 administering to said subject an effective amount the pharmaceutical
4 composition of claim 57.

1 62. A method of providing Growth Hormone therapy to a subject in need
2 of said therapy, said method comprising:
3 administering to said subject an effective amount the pharmaceutical
4 composition of claim 53.

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